

Features of Human Slit-1 predicted protein Co-ordinates refer to amino acid number.

| | | |
|-------------------------------------|-----------|-------------|
| Signal sequence: | 7-24 | |
| First amino-flanking sequence: | 28-59 | |
| First set of Leucine Rich Repeats: | 60-179 | (6 repeats) |
| First carboxy-flanking sequence: | 180-276 | |
| Second amino-flanking sequence: | 277-308 | |
| Second set of Leucine Rich Repeats: | 309-434 | (5 repeats) |
| Second carboxy-flanking sequence: | 435-501 | |
| Third amino-flanking sequence: | 502-533 | |
| Third set of Leucine Rich Repeats: | 534-660 | (5 repeats) |
| Third carboxy-flanking sequence: | 661-722 | |
| Fourth amino-flanking sequence: | 723-754 | |
| Fourth set of Leucine Rich Repeats: | 755-855 | (4 repeats) |
| Fourth carboxy-flanking sequence: | 856-917 | |
| First EGF repeat: | 918-952 | |
| Second EGF repeat: | 953-993 | |
| Third EGF repeat: | 994-1031 | |
| Fourth EGF repeat: | 1032-1071 | |
| Fifth EGF repeat: | 1072-1109 | |
| Spacer: | 1110-1116 | |
| Sixth EGF repeat: | 1117-1154 | |
| "99aa spacer": | 1155-1329 | |
| Seventh EGF repeat: | 1330-1366 | |
| Eighth EGF repeat: | 1367-1404 | |
| Ninth EGF repeat: | 1405-1447 | |
| Cysteine knot motif: | 1448-1525 | |

Leucine rich repeats (LRRs) are predicted by comparison with known proteins and by the presence of the core sequence: xxxFxxLxxLxxLxLxxNxIxxL, where x is any amino acid. In slit proteins, the LRRs are flanked by conserved sequences referred to as the amino- and carboxy- flanking regions. These flanking regions are found in other known proteins, but only in a few instances are both the amino- and carboxy- flank regions present in a single protein. The amino flank region is defined by the consensus: CPxxCx C[1-6x]GxxVDCxxxGL[2-4x] α Pxx α Pxdttx where x is any amino acid, [x] represents a variable number of amino acids and α is a hydrophobic residue. Lower case indicates a residue is not highly conserved at a particular position. The carboxy flank region is defined by the consensus: P β C γ Cx α [1-5x]W α [14-26x]RCxxPxxxxxxxx α xx α xxxxF[1-3x]Cs[3-17x] where β is W or a hydrophobic residue, γ is D or N and α is a hydrophobic residue.

Epidermal growth factor (EGF) repeats are predicted by the consensus: CxxxxCxnxC[6-9x] α CxCxxG α GxxCxxxxx.

The so called "99aa spacer" is actually ~200 amino acids in the *Drosophila* protein and 174 amino acids in Human Slit-1. This region shows homology to the G-loops of laminin A chains.

Cysteine knots are dimerisation domains defined by the presence of six cysteine residues between which disulphide bridges form. The only absolutely conserved residues are the six cysteines, and spacing between them is highly variable, apart from between cysteines 2 and 3, and 5 and 6: C[x]C[1-3x]GxC[x]C[x]CxC. The glycine between cysteines 2 and 3 is only present in a subset of cysteine knots. *Drosophila* slit and Human slit-1 both have an extra cysteine after cysteines 5 and 6: this may serve as an intermolecular bond.

Human Slit-1 gene displays the overall structure of the *Drosophila* gene, and amino acid conservation is found along the entire length of the protein (48% homology at the amino acid sequence excluding the signal sequence; see below). The Human gene has an extra LRR between LRR2 and LRR3 of the first set of LRRs; in the third set, the Human gene has an extra LRR between LRR3 and LRR4. The Human gene has two extra EGF repeats, on either side of the seventh EGF repeat in *Drosophila* slit.

Isolation of Human slit-1

Searching of the EST database revealed an EST, ab16g10.r1, with homology to the 99aa spacer region of *Drosophila* slit. This EST was used to probe a Human fetal brain library (Stratagene), and clones for Human slit-1 were isolated.

Amino acid identity between Drosophila Slit and Human Slit-1

| | | |
|-------------------------------------|--------|-------------------------------|
| First amino-flanking sequence: | 53% | |
| First set of Leucine Rich Repeats: | 52% | (54%, 67%, NA, 38%, 54%, 50%) |
| First carboxy-flanking sequence: | 42% | |
| Second amino-flanking sequence: | 50% | |
| Second set of Leucine Rich Repeats: | 60% | (54%, 58%, 67%, 71%, 50%) |
| Second carboxy-flanking sequence: | 62% | |
| Third amino-flanking sequence: | 56% | |
| Third set of Leucine Rich Repeats: | 49% | (46%, 46%, 42%, NA, 58%) |
| Third carboxy-flanking sequence: | 36% | |
| Fourth amino-flanking sequence: | 53% | |
| Fourth set of Leucine Rich Repeats: | 48% | (25%, 58%, 46%, 63%) |
| Fourth carboxy-flanking sequence: | 63% | |
| First EGF repeat: | 34% | |
| Second EGF repeat: | 46% | |
| Third EGF repeat: | 46% | |
| Fourth EGF repeat: | 35% | |
| Fifth EGF repeat: | 47% | |
| Spacer: | 22% | |
| Sixth EGF repeat: | 40% | |
| "99aa spacer": | 38% | |
| Seventh EGF repeat: | 11%/NA | |
| Eighth EGF repeat: | 44% | |
| Ninth EGF repeat: | 29%/NA | |
| Cysteine knot motif: | 34% | |

NA: not applicable due to absence of homologous repeat.
Figures for individual LRRs are shown in brackets.

| | | |
|-----|---|---------|
| 82 | DCNK RGLNTIP TSI PRFATQLLLSGNNISTV D LNSNIHVL | CE-Slit |
| 531 | DCTGRRLKEIP RDIP LHTTE LLLNDNELGR ISSDGLFGRL | D-Slit |
| 514 | DCSNQKLNK IPEHIPQYTAELRLNNNEFTVLEATGI FKKL | H-Slit1 |
| 122 | ENLEXLDLSNNHITFINDKSF EKLSKLRELXLND | CE-Slit |
| 571 | PHLVKLELKR NQLTGIEPN AFEGASHIQELQLGENKIKEI | D-Slit |
| 554 | PQLRKINFSNNKITDIEEGAFEGASGVNEILLTSNRL ENV | H-Slit1 |
| 1 | EGAFNGAASVOELMLTG NQLETV | H-Slit2 |
| 611 | SNKMF - - - - - LGLHQLKTLN | D-Slit |
| 594 | QH KMFKG - LESLKTLM LRSNRITCVGNDSFI GLSSVRLLS | H-Slit1 |
| 24 | HGRGFRGGISGLKTLM LRSNLI GCVSNDTFA GLSSVRLLS | H-Slit2 |
| 626 | LYDNQISCVMPGSFEHLNSLTS LNLA SNPFNCNCHLAW - F | D-Slit |
| 633 | LYDNQITTTVA PGAFDTLHSLSTLNL L ANPFNCNCYLA W - L | H-Slit1 |
| 64 | LYDNRI TTTIT PGAF TTVLSLSTI NLLSNPFNCNCHL GAGL | H-Slit2 |
| 665 | AECVRKKSLNGGAARCGAPSKVRDVQIKDLPHSEFKCSSE | D-Slit |
| 672 | GEWLRKKRIVTGNPRCQKPYFLKEIPIQDVAIQDFTCD DG | H-Slit1 |
| 104 | GKWLRKKRRIVS GNPRCQKPF FLKEIPIQGVGHPI | H-Slit2 |
| 1 | SNKNLTSFPSRI PFD | CE-Slit |
| 705 | NSE - GCLGDGYCPPSCTCTGT VVAACSRNQ LKEIPRGIPAE | D-Slit |
| 712 | HDDNSCSPLSRCPT ECTCLD TVVRC SNKGLKVLPKGIPRD | H-Slit1 |
| 16 | TEELYLDANYINEIPAH DINRLYS LTKLDLSHNRLISLEN | CE-Slit |
| 744 | TSELYLESNEIEQIHYERIRHLRSLTRL DLSNNQITILSN | D-Slit |
| 752 | VT ELYLDGNQFTLVPKF - LS NYKHLTLIDLSNNRISTLSN | H-Slit1 |
| 56 | NTFSNLTR LSTLIISYNKLRCLQPLAFNGLNALRI LSLHG | CE-Slit |
| 784 | YT FANLTK LSTLIISYNKLOCLQRHALSGLNNLRV VSLHG | D-Slit |
| 791 | QSFSNM TQLLT LILSYNRLRCIPPRTFDGLKSLRL LSLHG | H-Slit1 |
| 96 | NDISFLPOSASF SNLT SITHIAVGSNSLYCDCNM AWFSKWI | CE-Slit |
| 824 | NRISM LPEGSEFDLKSLTHIALGSNPLYCDCGLKWFSDWI | D-Slit |
| 831 | NDISVVPEGAFNDLSALSHLAIGANPLYCDCNMHQLSDWV | H-Slit1 |
| 436 | KSKFI EAGIARCEY PNTVSNQLLLTAQPYQFTCD SKVPTK | CE-Slit |
| 864 | KLDYVEPGIARCAEPEQM KDKLILSTPSSSFVCRGRVRND | D-Slit |
| 871 | KSEYKEPGIARCAGPGEMADKLLLTTPSKKFTCQGPVDVN | H-Slit1 |
| 176 | LATKCDLCLNSPCKNNNAICETTS SRKYTCNCTPGFYGVHC | CE-Slit |
| 904 | ILAKCNACFEQPCQNQAQCVALPQREYQCLCQPGYHGKH C | D-Slit |
| 911 | ILAKCNPCLSNPNCKNDGTCNSDPVDFYRCTCPYGFKGQDC | H-Slit1 |
| 216 | ENQIDACYGSPCLNNATCKV - - AQAGR FN CYCNKGFE G DY | CE-Slit |
| 944 | EFMIDACYGNPCRNNATCTVLE - - EGRFS CQCAPGYTGAR | D-Slit |
| 951 | DVP I HACISNPCKHGGTCHLKEGEEDGFWCICADGFEGEN | H-Slit1 |
| 254 | CEKNIDDCV - NSK CENG GKCVDLVRFCS EELKNFQSFOIN | CE-Slit |
| 982 | CETNIDDC LGEIKCQNNATCID - - - - - GVE | D-Slit |
| 991 | CEVNVDDC - EDND CENNSTCVD - - - - - GIN | H-Slit1 |

293 SYRCD CPM EYE GKHCED KLEYCTKK LNPCEN NGKCIP IN G CE-Slit
1007 SYKCEC QPGFS GEFCDTK IQFCSP EFNPCAN GA KCM D H FT D-Slit
1015 NYTCLCPPEYT GELCEEK LDFCAQDLNPCQHDSKCI LTPK H-Slit1
1 DPLPV M-Slit2

333 SYSCMCSPGFTGN NCE TNIDDCKNVE CQNGGS SCVDGILSY CE-Slit
1047 HYS CDCQAGFHGT NCTDNIDDCQNHMCONGGT CVDGIN DY D-Slit
1055 GFKC DCTPGYVGEHC D I D F D D C Q D N K C K N G A H C T D A V N G Y H-Slit1
1 NNDDC VGHKCRHGAQCVDDEVNGY M-Slit1
1 WPRCECM PGYAGDNCSENQDDCRDHR CQNGAQC MDEVNSY H-Slit2
6 HHRCECH LGYTGDNCSENODDC KDHKCQNGAQCVDDEVNSY M-Slit2

373 DCLCRPGYAGQYCEIPPM MDHEYQKTDACQQSACGQG-EC CE-Slit
1087 QCRCPDDYT GK YCE GHNMISMMYPQTSPCQNH ECKHGV-C D-Slit
1095 TCICPEGYSGLFCE FSP--PMVLPRTSPCDNFDCQNGAQC H-Slit1
24 TCICP QGFSGGLFCE HPP--PMVL LQTSPCDQYECQNGAQC M-Slit1
41 SCLCAEGYSGLCEIPP--HLPAPK-SPCEGTECQNGANC H-Slit2
46 ACLCV EGYSGQLCEIPP-- -- -- APR-SSCEGTECQNGANC M-Slit2

412 VASQN-S SDF TCKCHEGFS GSPSCDROMSVGFKNPGAYLAL CE-Slit
1126 FQPNAGGSDYL CRCHPGYT GKWCEYLTSISFVHNNSFVEL D-Slit
1133 IVRINEP---ICQCLPGYQ GGEKCEKLVSVNFINKESY LQI H-Slit1
62 IVVQQEP---TCRC PPGFAAGPRCEKLI TVN FVGKDSYVEL M-Slit1
78 VDQGNRP---VCQCLPGFGGPECEKLLSVNFVDRD TYLQF H-Slit2
80 VDQGS RP---VCQCLPGFGGPECEKLLSVNFVDRD TYLQF M-Slit2

451 DPLAS--DGTITMTLRTTSKIGILLYY GDDH FVSAELYD G CE-Slit
1166 EPLRTRPEANVTIVFS SAEQNGILMYD GQDAHLA VELFNG D-Slit
1170 PSAKVRPQT NITLOIA TDEDSGILLYKGD KDHIAVELYRG H-Slit1
99 ASAKVR M-Slit1
115 TDLQNW XRXNITLQVF FTAEDNGILLYNGGNDHIAV XLYXG H-Slit2
117 TDLQNW PRANITLOVSTAEDNGILLYNGDNDHIAVELY M-Slit2

489 RVKLVYYIGNFPASHMYSSVKVNDGLPHRISIRTSERKCF CE-Slit
1206 RIRVSYDVGNHPVSTHYSFEMVADGKYH AVELLA I KKNFT D-Slit
1210 RVRAS YDTGSHPASAIYSVETINDGNFHI VELLALDQSLS H-Slit1
155 HVRFSY H-Slit2

529 LQIDKNPVQ IVENSGKSDQLITK GKEMLYIGGLPIEKSQD CE-Slit
1246 LRVDRGLARS IINEEGSNDYL--KLTTPMFLGGLPVDP AQO D-Slit
1250 LSV DGGNFK IITNL SKQSTL--NFDSPLYVGGMPG KSNVA H-Slit1
1 ILDVA M-Slit1

569 AKRRFHVKNSESLKGCISSITINEVPINLQQALENVNTEQ CE-Slit
1284 AYKNWQIRNLTSFKGCMKEVWINHKLVD FGN AQRRQKITP D-Slit
1288 SLRQAPGQNGTSF HGCIRNLYINSELQDFQKVPMQTGILP H-Slit1
6 SLRQAPGE NGTSF HGCIRNLYINSELQDFRKM PMOTGILP M-Slit1

609 SC-----SATVNF----- CE-Slit
1324 GCAL-----LEGEQQEEEDDEQDFMDE-----TPHIKEEP D-Slit
1328 GCEPCHKKVCAHGT CQPS SQA GFTCECQEGWMGPLCDQRT H-Slit1
46 GCEPCHKKVCAHGC CQPS SQS GFTCECEEGWMGPLCDQRT M-Slit1

TABLE 4

Alignment of Drosophila Slit and Human Slit-1

| | | |
|-----|---|---------|
| 1 | M A A P S R T T L M P P P F R L Q L R L - L I L P I L L L L R H D A V H A E P Y | D-Slit |
| 1 | M R G V G W Q - - - - - M L S L S L G L V L A I L - - - - - | H-Slit1 |
| 40 | S G G F G S S A V S S G G L G S V G I H I P G G V G V I T E A R C P R V C S C | D-Slit |
| 21 | - - - - - - - - - - - - - - - - - N K V A P Q A C P A Q C S C | H-Slit1 |
| 80 | T G L N V D C S H R G L T S V P R K I S A D V E R L E L Q G N N L T V I Y E T D | D-Slit |
| 35 | S G S T V D C H G L A L R S V P R N I P R N T E R L D L N G N N I T R I T K T D | H-Slit1 |
| 120 | F Q R L T K L R M L Q L T D N Q I H T I E R N S F Q D L V S L E R L - - - - - | D-Slit |
| 75 | F A G L R H L R V L Q L M E N K I S T I E R G A F Q D L K E L E R L R L N R N H | H-Slit1 |
| 154 | - - - - - - - - - - - - - - - - D I S N N V I T T V G R R V F K G A Q S L R | D-Slit |
| 115 | L Q L F P E L L F L G T A K L Y R L D L S E N Q I Q A I P R K A F R G A V D I K | H-Slit1 |
| 176 | S L Q L D N N Q I T C L D E H A F K G L V E L E I L T L N N N N L T S L P H N I | D-Slit |
| 155 | N L Q L D Y N Q I S C I E D G A F R A L R D L E V L T L N N N N I T R L S V A S | H-Slit1 |
| 216 | F G G L G R L R A L R L S D N P F A C D C H L S W L S R F L R S A T R L A P Y T | D-Slit |
| 195 | F N H M P K L R T F R L H S N N L Y C D C H L A W L S D W L R K R P R V G L Y T | H-Slit1 |
| 256 | R C Q S P S Q L K G Q N V A D L H D Q E F K C S G L T E - H A P M - - - E C G A | D-Slit |
| 235 | Q C M G P S H L R G H N V A E V Q K R E F V C S D E E E G H Q S F M A P S C S V | H-Slit1 |
| 292 | E N S C P H P C R C A D G I V D C R E K S L T S V P V T L P D D T T D V R L E Q | D-Slit |
| 275 | L H - C P A A C T C S N N I V D C R G H G L T E I P T N L P E T I T E I R L E Q | H-Slit1 |
| 332 | N F I T E L P P K S F S S F R R L R R I D L S N N N I S R I A H D A L S G L K Q | D-Slit |
| 314 | N T I K V I P P G A F S P Y K K L R R I D L S N N Q I S E L A P D A F Q G L R S | H-Slit1 |
| 372 | L T T L V L Y G N K I K D L P S G V F K G L G S L R L L L L N A N E I S C I R K | D-Slit |
| 354 | L N S L V L Y G N K I T E L P K S L F E G L F S L Q L L L L N A N K I N C L R V | H-Slit1 |
| 412 | D A F R D L H S L S L L S L Y D N N I Q S L A N G T F D A M K S M K T V H L A K | D-Slit |
| 394 | D A F Q D L H N I L N L L S L Y D N K L Q T I A K G T F S P L R A I Q T M H L A Q | H-Slit1 |
| 452 | N P F I C D C N L R W L A D Y L H K N P I E T S G A R C E S P K R M H R R R I E | D-Slit |
| 434 | N P F I C D C H L K W L A D Y L H T N P I E T S G A R C T S P R R L A N K R I G | H-Slit1 |
| 492 | S L R E E K F K C S - W G E L R M K L S G E C R M D S D C P A M C H C E G T T V | D-Slit |
| 474 | Q I K S K K F R C S G T E D Y R S K L S G D C F A D L A C P E K C R C E G T T V | H-Slit1 |
| 531 | D C T G R R L K E I P R D I P L H T T E L L L N D N E L G R I S S D G L F G R L | D-Slit |
| 514 | D C S N Q K L N K I P E H I P Q Y T A E L R L N N N E F T V L E A T G I F K K L | H-Slit1 |
| 571 | P H L V K L E L K R N Q L T G I E P N A F E G A S H I Q E L Q L G E N K I K E I | D-Slit |
| 554 | P Q L R K I N F S N N K I T D I E E G A F E G A S G V N E I L L T S N R L E N V | H-Slit1 |
| 611 | S N K M F L G L H Q L K T L - - - - - - - - - - - - - - - N L | D-Slit |
| 594 | Q H K M F K G L E S L K T L M L R S N R I T C V G N D S F I G L S S V R L L S L | H-Slit1 |
| 627 | Y D N Q I S C V M P G S F E H L N S L T S L N L A S N P F N C N C H L A W F A E | D-Slit |
| 634 | Y D N Q I T T V A P G A F D T L H S L S T L N L L A N P F N C N C Y L A W L G E | H-Slit1 |

| | | |
|------|---|---------|
| 667 | C V R K K S L N G G A A R Q A P S K V R D V Q I K D L P H S K C S S E N S | D-Slit |
| 674 | W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D T C D D G M D | H-Slit1 |
| 707 | E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E T S | D-Slit |
| 714 | D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D Y T | H-Slit1 |
| 746 | E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N Y T | D-Slit |
| 754 | E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S M Q S | H-Slit1 |
| 786 | F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G N R | D-Slit |
| 793 | F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G N D | H-Slit1 |
| 826 | I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I K L | D-Slit |
| 833 | I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q M L S D W V K S | H-Slit1 |
| 866 | D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D I L | D-Slit |
| 873 | E Y K E P G I A R C A G P G E H A D K L L L T T P S K K F T C Q G P V D V N I L | H-Slit1 |
| 906 | A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C E F | D-Slit |
| 913 | A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C D V | H-Slit1 |
| 946 | M I D A C Y G N P C R N N A T C T V L E - - E G R F S C Q C A P G Y T G A R C E | D-Slit |
| 953 | P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N C E | H-Slit1 |
| 984 | T H I D D C L G E I K C Q N H A T C I D G V E S Y K C E C Q P G F S G E F C D T | D-Slit |
| 993 | V H V D D C - E D N D C E N H S T C V D G I N N Y T C L C P P E Y T G E L C E E | H-Slit1 |
| 1024 | K I Q F C S P E F H P C A N G A K C M D H F T H Y S C D C Q A G F H G T N C T D | D-Slit |
| 1032 | K L D F C A Q D L N P C Q H D S K C I L T E K G F K C D C T P G Y V G E H C D I | H-Slit1 |
| 1064 | N I D D C Q N H M C Q N G G T C V D G I N D Y Q C R C P D D Y T G K Y C E G H N | D-Slit |
| 1072 | D F D D C Q D N K C K N G A H C T D A Y H G Y T C I C P E G Y S G L F C E F S P | H-Slit1 |
| 1104 | M I S M M Y P Q T S P C Q N H E C K H G V - C F Q P N A Q G S D Y L C R C H P G | D-Slit |
| 1112 | - - P M V L P R T S P C D H F D C Q N G A Q C I - - - V R I N E P I C Q C L P G | H-Slit1 |
| 1143 | Y T G K W C E Y L T S I S F V H N N S F V E L E P L R T R P E A N V T I V F S S | D-Slit |
| 1147 | Y Q G E K C E K L V S V N F I N K E S Y L Q I P S A K V E P Q T H I T L Q I A T | H-Slit1 |
| 1183 | A E Q N G I L M Y D G Q D A H L A V E L F N G R I R V S Y D V G N H P V S T M Y | D-Slit |
| 1187 | D E D S G I L L Y K G D K D H I A V E L Y R G R V R A S Y D T G S H P A S A I Y | H-Slit1 |
| 1223 | S F E M V A D G K Y H A V E L L A I K K N F T L R V D R G L A R S I I N E G S N | D-Slit |
| 1227 | S V E T I N D G N F H I V E L L A L D Q S L S L S V D G G N P K I I T N L S K Q | H-Slit1 |
| 1263 | D Y L K L T T P M F L G G L P V D P A Q Q A Y K N W Q I R N L T S F K G C M K E | D-Slit |
| 1267 | S T L N F D S P L Y V G G M P G K S N V A S L R Q A P G Q N G T S F H G C I R N | H-Slit1 |
| 1303 | V W I N H K L V D F G N A Q R Q Q K I T P G C A L - - - - L E G E Q Q E E E D D | D-Slit |
| 1307 | L Y I N S E L Q D F Q K V P M O T G I L P G C E P C H K K V C A H G T C Q P S S | H-Slit1 |
| 1339 | E Q D F M D E - - - - - T P H I K E E P V D P C L E N K C R R G S R C V P N S | D-Slit |
| 1347 | Q A G F T C E C Q E G W M G P L C D Q R T N D P C L G N K C V H G T - C L P I N | H-Slit1 |

1373 N A R D G Y Q C K C K H G Q S R Y C D Q G E G S T E P - - - - - D-Slit
1386 A F - - S Y S C K C L E G H G G V L C D E E E D L F N P C Q A I K C K H G K C R H-Slit1

1401 - - - - - P T V T A A S - - - - - T C R K E Q V R E Y Y T E N D - D-Slit
1424 L S G L G Q P Y C E C S S G Y T G D S C D R E I S C R G E R I R D Y Y Q K Q Q G H-Slit1

1423 - - - C R S R Q P L K Y A K C V G G C - G N Q C C A A K I V R R R K V R M V C S D-Slit
1464 Y A A C Q T T K K V S R L E C R G G C A G G Q C C G P L R S K R R K Y S F E C T H-Slit1

1459 N N R K Y I K N L D I V R K C G C T K K C Y D-Slit
1504 D G S S F V D E V E K V V K C G C T R - C V S H-Slit1

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TABLE 5(A)

Hybridisation Probes for regions of Human Slit-1

Hybridisation Probe for the first Leucine rich repeat region

| | |
|---|-----|
| TGCCCGGCGCAGTGCTCTTGCTCGGGCAGCACAGTGGACTGTACGGGCTGGCGCTGCGCAGCGTGCCAGGAAT | 75 |
| ATCCCCCGCAACACCGAGAGACTGGATTTAAATGGAAATAACATCACAGAATTACGAAGACAGATTTTGCTGGT | 150 |
| CTTAGACATCTAAGAGTTCTTCAGCTTATGGAGAATAAGATTAGCACCATTGAAAGAGGAGCATTCCAGGATCTT | 225 |
| AAAGAACTAGAGAGACTGCGTTTAAACAGAAATCACCTTCAGCTGTTTCTGAGTTGCTGTTTCTTGGGACTGCG | 300 |
| AAGCTATACAGGCTTGATCTCAGTGAACCAAAATTCAGGCAATCCCAAGGAAAGCTTTCCGTTGGGCGAGTTGAC | 375 |
| ATAAAAAATTTGCAACTGGATTACAACCAGATCAGCTGTATTGAAGATGGGGCATTTCAGGCTCTCCGGGACCTG | 450 |
| GAAGTGTCACTCTCAACAATAACAACATTACTAGACTTTCTGTGGCAAGTTTCAACCATATGCCTAAACTTAG | 525 |
| ACTTTTCGACTGCATTCAACAACCTGTATTGTGACTGCCACCTGGCCTGGCTCTCCGACTGGCTTCGCAAAAGG | 600 |
| CCTCGGGTTGGTCTGTACACTCAGTGTATGGGCCCCCTCCACCTGAGAGGCCATAATGTAGCCGAGGTTCAAAAA | 675 |
| CGAGAATTGTCTCGAGTGATGAGGAAGAAGGTACCAGTCATTATGGCTCCTTCTGTAGTGTGTTTGCAC | 747 |

82-828

Hybridisation Probe for the second Leucine rich repeat region

| | |
|--|-----|
| TGCCCTGCCGCTGTACCTGTAGCAACAATATCGTAGACTGTCTGGGAAAGGTCTCACTGAGATCCCCACAAAT | 75 |
| CTTCCAGAGACCATCACAGAAATACGTTTGAACAGAACACAATCAAAGTCATCCCTCCTGGAGCTTTCTCACCA | 150 |
| TATAAAAGCTTAGACGAATTGACCTGAGCAATAATCAGATCTCTGAAGTTGACCCAGATGCTTTCCAGGACTA | 225 |
| CGCTCTCTGAATCACTTGTCTCTATGGAATAAAATCACAGAACTCCCCAAAGTTTATTGAAGGACTGTTT | 300 |
| TCCTTACAGCTCCTATTATTGAATGCCAACAGATAAACTGCCCTTCGGGTAGATGCTTTTCAGGATCTCCACAAC | 375 |
| TGAACCTTCTCTCCCTATATGACAACAAGCTTCAGACCATCGCCAAGGGGACCTTTTCACTCTTCGGGCCATT | 450 |
| CAAACTATGCATTGCGCCAGAACCCCTTTTATTGTGACTGCCATCTCAAGTGGCTAGCGGATTATCTCCATACC | 525 |
| AACCCGATTGAGACCAGTGGTGGCCGTTGCAAGCCCGCCGCTGGCAACAAAAGAATTGGACAGATCAAA | 600 |
| AGCAAGAAATTCGGTTGTTTCAGGTACAGAAGATTATCGATCAAAATTAAGTGGAGACTGCTTTGCGGATCTGGCT | 675 |

829-1503

Hybridisation Probe for the third Leucine rich repeat region

| | |
|--|-----|
| TGCCCTGAAAAGTGTCTGCTGTGAAGGAACCAAGTGAATTTGCTCTAATCAAAAGCTCAACAAAATCCCGGAGCAC | 75 |
| ATTCCCCAGTACACTGCAGAGTTGCGTCTCAATAATAATGAATTTACCGTGTGGAAGCCACAGGAATCTTTAAG | 150 |
| AAACTTCCTCAATTACGTAAATAAACTTTAGCAACAATAAGATCACAGATATTGAGGAGGGAGCATTGGAAGGA | 225 |
| GCATCTGGTGTAATGAAATACTTCTTACGAGTAATCGTTTGGAAAATGTGCAGCATAAGATGTTCAAGGGATTG | 300 |
| GAAAGCCTCAAACTTTGATGTTGAGAAGCAATCGAATAACCTGTGTGGGGAATGACAGTTTCATAGGACTCAGT | 375 |
| TCTGTGCGTTTGCTTTCTTTGTATGATAATCAAAATTACTACAGTTGCACAGGGGCATTTGATACTCTCCATTCT | 450 |
| TTATCTACTCTAAACCTCTTGGCZAATCCTTTTAACTGTAAGTGTACCTGGCTTGGTTGGGAGAGTGGCTGAGA | 525 |
| AAGAAGAGAATTGTACGGGGAATCCTAGATGTCAAAACCATACTTCCTGAAAGAAATACCCATCCAGGATGTG | 600 |
| GCCATTACAGGACTTCACTTGTGATGACGGAAATGATGACAATAGTTGCTCCCCACTTTCTCGC | 663 |

1504-2166

Hybridisation Probe for the fourth Leucine rich repeat region

| | |
|--|-----|
| TGTCCTACTGAATGTACTTGCTTGGATACAGTCGTCGATGTAGCAACAAGGGTTTGAAGGTCTTGCCGAAAGGT | 75 |
| ATTCCAAGAGATGTACAGAGTTGTATCTGGATGGAAACCAATTTACACTGGTTCCCAAGGAACCTCTCCAATAC | 150 |
| AAACATTTAACTTATAGACTTAAGTAACAACAGAATAAGCACGCTTTCTAATCAGAGCTTCAGCAACATGACC | 225 |
| CAGCTCCTCACCTTAATCTTAGTTACAACCGTCTGAGATGTATTCCTCCTCGCACCTTTGATGGATTAAAGTCT | 300 |
| CTTCGATTACTTTCTCTACATGGAAATGACATTTCTGTTGTGCTGAAGGTGCTTTCAATGATCTTTCTGCATTA | 375 |
| TCACATCTAGCAATTGGAGCCAACCTCTTTACTGTGATTGTAACATGCAGTGGTTATCCGACTGGGTGAAGTCG | 450 |
| GAATATAAGGAGCCTGGAATTGCTCGTTGTGCTGGTCCGAGAAATGGCAGATAAACTTTTACTCACAACCTCC | 525 |
| TCCAAAAATTTACCTGTCAAGGTCTGTGGATGTCAATATTCTAGCTAAGTGTAAACCC | 585 |

2167-2751

Hybridisation Probe for EGF repeats one to five

| | |
|--|-----|
| TGCTTATCAAATCCGTGTAAAAATGATGGCACATGTAATAGTGATCCAGTTGACTTTTACCGATGCACCTGTCCA | 75 |
| TATGGTTTCAAGGGGCGAGACTGTGATGTCCCAATTCATGCTGCATCAGTAACCATGTAAACATGGAGGAAT | 150 |
| TGCCACTTAAAGGAAGGAGAAGAAGATGGATTCTGGTGTATTGTGCTGATGGATTTGAAGGAGAAAATTGTGA | 225 |
| GTCAACGTTGATGATTGTGAAGATAATGACTGTGAAAATAATTCTACATGTGTCGATGGCATTAAATAACTACACA | 300 |
| TGCTTTGCCACCTGAGTATACAGGTGAGTTGTGTGAGGAGAAGCTGGACTTCTGTGCCAGGACCTGAACCCC | 375 |
| TGCCAGCAGATTCAAAGTGCATCCTTAACCTCAAAGGGATTCAAATGTGACTGCACACCAGGGTACGTAGGTGAA | 450 |
| CACTGCGACATCGATTTTACGACTGCCAAGACAACAAGTGAACCGAGCCCACTGCACAGATGCAGTGAAC | 525 |
| GGCTATACGTGCATATGCCCCGAAGGTTACAGTGGCTTGTCTGTGAGTTT | 576 |

2752-3323

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TABLE 5(B)

Hybridisation Probe for the sixth EGF repeat and preceding spacer region

TCTCCACCCATGGTCTCCCTCGTACCAGCCCTGTGATAATTTTGATTGTCAGAATGGAGCTCAGTGTATCGTC 75
AGAATAAATGAGCCAATATGTCACTGTTTGCTGGCTATCAGGGAGAAAAGTGTGAAA 134

3028-3161

Hybridisation Probe for the 99aa spacer/G-loop region

ATTGGTTAGTGTGAATTTTATAACAAAGAGTCTTATCTTCAGATTCTTCAGCCAAGGTTCCGCCTCAGACGAA 75
CATAACACTTCAGATTGCCACAGATGAAGACAGCGGAATCCTCCTGTATAAGGTTGACAAAGACCATATCGCGGT 150
AGAACTCTATCGGGGGCGTGTTCGTGCCAGCTATGACACCGGCTCTCATCCAGCTTCTGCCATTTACAGTGTGGA 225
GACAATCAATGATGGAACCTCCACATTGTGGAACCTACTGCTTGGATCAGAGTCTCTCTTTGTCCGTGGATGG 300
TGGAACCCCAAATCATCACTAATCTGTCAAAGCAGTCCACTCTGAATTTTGACTCTCCACTCTATGTAGGAGG 375
CATGCCAGGGAAGAGTAACGTGGCATCTCTGCCCCAGGCCCCCTGGGCAGAACGGAACAGCTTCCACGGCTGCAT 450
CCGGAACCTTTACATCAACAGTGAGCTGCAGGACTTCCAGAAGGTGCGCATGCAACAGGCATTTTGCTGGCTGT 526

3162-3987

Hybridisation Probe for EGF repeats seven to nine

GAGCCATGCCACAAGAAGGTGTGTGCCCATGGCAATGCCAGCCAGCAGCCAGGCAGGCTTCACCTGCGAGTGC 75
CAGGAAGGATGGATGGGGCCCTCTGTGACCAACCGACCAATGACCCTTGCTTGGAAATAAATGCGTACATGGC 150
ACCTGCTTGCCCATCAATGCGTTCTCCTACAGCTGTAACTGCTTGGAGGGCCATGGAGGTGTCTCTGTGATGAA 225
GAGGAGGATCTGTTTAACCCATGCCAGGCGATCAAGTCAAGCATGGGAAGTGACAGGCTTTCAGGTCTGGGGCAG 300
CCCTACTGTGAATGCAGCAGTGGATACACGGGGACACTGTGATCGAGAAATC 353

3988-4341

Hybridisation Probe for the cysteine knot region

TCTGTGCGAGGGGAAAGGATAAGAGATTATTCACAAAACAGCAGGGCTATGCTGCTTGCCAAACAACCAAGAAG 75
GTGTCCCGATTAGAGTGCAGAGGTGGGTGTGACGAGGGCAGTGTGTGGACCGCTGAGGAGCAAGCGCGGAAA 150
TACTCTTTCGAATGCACTGACGGCTCTCTTTGTGGACGAGGTTGAGAAAGTGGTGAAGTGCGGCTGTACGAGG 225
TGTGTGTCC 234

4342-4575

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PCR Primers for regions of Human Slit-1**PCR Primers for the first Leucine rich repeat region**

Forward: 5' TGCCCGGCGCAGTGTCTTGCTCGGGCAGC 3' 82-111
 Reverse: 5' GTGCAAAACACTACAAGAAGGAGCCATAAA 3' 799-120 (82)

PCR Primers for the second Leucine rich repeat region

Forward: 5' TGCCCTGCCGCTGTACCTGTAGCAACAAT 3' 824-858
 Reverse: 5' AGCCAGATCCGCAAAGCAGTCTCCACTTAA 3' 1174-1572 RL

PCR Primers for the third Leucine rich repeat region

Forward: 5' TGCCCTGAAAAGTGTGCTGTGAAGGAACC 3' 1504-1533
 Reverse: 5' GCGAGAAAGTGGGAGCAACTATTGTCATC 3' 2137-2166

PCR Primers for the fourth Leucine rich repeat region

Forward: 5' TGTCTACTGAATGTACTTGCTGGATACA 3' 2167-2196
 Reverse: 5' GGGTTACACTTAGCTAGAAATTGACATC 3' 2722-2751

PCR Primers for EGF repeats one to five

Forward: 5' TGCCTATCAAATCCGTGTAAAAATGATGGC 3' 2752-2781
 Reverse: 5' AAATCACAGAACAGCCACTGTAACCTTC 3' 3248-3327

PCR Primers for the sixth EGF repeat and preceding spacer region

Forward: 5' TCTCCACCCATGGTCCCTCCCTCGTACCAGC 3' 3329-3357
 Reverse: 5' TTTTCACACTTTTCTCCCTGATAGCCAGGC 3' 3432-3461

PCR Primers for the 99aa spacer/G-loop region

Forward: 5' ATTGGTTAGTGTGAATTTTATAAACAAAGA 3' 3462-3491
 Reverse: 5' ACAGCCAGGCAAAATGCCTGTTTGCATCGG 3' 3758-3787

PCR Primers for EGF repeats seven to nine

Forward: 5' GAGCCATGCCACAAGAAGGTGTGTGCCCAT 3' 3988-4017
 Reverse: 5' GATTTCTCGATCACAGCTGTCCCGTGTAT 3' 4312-4341

PCR Primers for the cysteine knot region

Forward: 5' TCTTGTCGAGGGGAAAGGATAAGAGATTAT 3' 4712-4731
 Reverse: 5' GGACACACCTCGTACAGCCGCACTTCAC 3' 4546-4575

007620" 54204560